Amendments to the Specification:

Please replace the Title with the following Title:

"METHODS FOR DETECTING TROPHOBLAST MALIGNANCY BY hCG ASSAY"

Please amend the paragraph beginning at page 76, line 13, and insert Table 5 as follows:

A variety of hCG isoforms were employed to characterize the new antibodies described in this report and the nomenclature and characteristics of each of the reagents employed is summarized in ~~Figure 11~~ Table 5. The carbohydrate groups in these hCG isoforms as well as the percent nicking were analyzed in an earlier study (26) and are directly relevant for defining the nature of these new antibodies in this report.

Table 5: Characteristics of the reagents used to define antibody specificity. The peptide and carbohydrate structures of the reagents used were determined earlier (26). The % nicked β-subunit refers to the proportion of molecules with cleavages (missing peptide bonds) in the region β43 to β48. The % tetrasaccharide core is the proportion of O-linked oligosaccharides with tetrasaccharide (vs disaccharide) core structure, and the % sialic acid is the proportion of O-linked structures with antennae terminated by sialic acid residues. The proportion of triantennary N-linked oligosaccharides on β-subunit is given, as is the corresponding % sialic acid.

Name	Source	N-sialic acid[a]	O-sialic acid[b]	% triantennary N-linked on β	% tetrasaccharide O-linked core	% β nicking
814 hCG	CR 127 hCG[c]	95	66	5	19	19
C5 chorioCG	chorio-carcinoma	95	58	48	100	100
M4 mole CG	mole pregnancy	120	49	30	20	98
813 hCGn	CR 127 hCG	nd[d]	nd	nd	nd	80
C7 chorioCG	chorio-carcinoma	68	53	48	69	3
P8 hCG	pregnancy	94	73	21	13	0
M4 mole β	mole pregnancy	120	49	30	20	98
C5 chorio β	C5 chorioCG	95	58	48	100	100
CR 129 β	CR 129 hCG	96	63	11	17	19
HLH I-1	pit hLH	nd	na	na	na	na
M1A	mole pregnancy	98.5	na	16.5	<15% CTP[e]	24

[a]percent sialic acid residues per sugar chain, N-linked on β
[b]percent sialic acid residues per sugar chain, O-linked on β
[c]The CR series of hCG reference preparations was made at Columbia Univeristy and distributed internationally as reference materials for purified hCG. CR 119 is also known as the 3rd international immunoassay reference preparation for hCG
[d]ND is not done; NA is not applicable for that reagent
[e]Less than 15% of the beta COOH-terminal region is present in this preparation

G1 cont

Please amend the paragraph beginning at page 76, line 22, and insert Table 6, as follows:

Two antibodies designated B151 and B152 were selected by the use of radiolabeled hCG isoforms, chorioCG C5 and pregnancy hCG CR 127. Each displayed preferential binding to C5 as compared to CR 127 since this was the selection criterion. However, upon performing liquid phase immunoassays and calculating affinity constants, it was clear that these two antibodies were very different in specificity (~~Figure 12~~ Table 6). It was found that antibody B151 had one order of magnitude higher affinity both for C5, which is nicked and hyperglycosylated choriocarcinoma hCG, and for CR 127 hCGn (813) as compared to CR 127 hCG or nick-free CR 127(814) (see ~~Figure 11~~ Table 5 for reagent descriptions). B151 was clearly an antibody with a strong preference for binding to various forms of nicked hCG. Antibody B152 was different in that although it displayed one order of magnitude preference for C5 hCG over CR 127 hCG, it recognized the nicked and non-nicked forms of CR 127 hCG, hCG derived from normal pregnancies, to an equal extent.

G2

Table 6: Affinity constants[a] determined by liquid phase competition assays using C5 as tracer ligand.

	Competitors			
Antibody	C5 chorioCG	Nicked hCG CR 127 (813)	Parent CR 127 hCG[b]	Nick-free hCG CR 127 (814)
B151	4.4×10^8	3.8×10^8	4.2×10^7	1.3×10^7
B152	3.5×10^9	5.4×10^7	4.7×10^7	5×10^7

[a]Ka as L/M
[b]hCG CR 127 is an NIH-distributed hCG reference preparation produced at Columbia University

Please amend the paragraph beginning at page 77, line 27, and insert Tables 7A and 7B as follows:

A variety of two site antibody formats were tested. ~~Figure 13~~ Table 7 displays these results. It is apparent that B151 cannot bind simultaneously with antibodies (designated by us as site IV) (27) to the beta subunit and beta subunit core (B201 and B204) nor with antibodies directed towards the determinant which exists in heterodimeric hCG as represented by antibody B109 (site III, to which A109 also belongs) (27). In contrast, a general beta antibody which binds to the most common and potent hCG antigenic site previously designated by us as site II (B108 or B207) binds well simultaneously with both B151 and B152 antibodies. B152 binds simultaneously to all antibodies tested except for those to the beta COOH-terminal region (CTP) (28) in contrast to B151 which binds well to CTP antibodies. B151 may represent a newly revealed hCG epitope which only exists on nicked hCG as reported in this manuscript.

G3

Table 7A: Relative cross-reactivities of two site assay using B151 as capture antibody.

Ligand	B207[a,d]	B204[a]	B201[a]	B108[a]	B109[a]	A109[a]	CTP104
C5	100%	<[c]	<	100%	<	<	100%
813 CR 127 hCGn	100%	<	<	100%	<	<	47%
814 CR 127 hCG	12%	<	<	37%	<	<	14%
hCGβ	2%	<	<	2%	<	<	<
C5β	5%	<	<		<	<	<
hCGβ core	<	<	<		<	<	<
hLH	2%	<	<	3%	<	<	<
hLHβ	5%	<	<		<	<	<
hCGα	<	<	<	3%	<	<	<
Max binding[b]	50%	0%	0%	13%	0%	0%	83%

[a]labeled detection antibodies
[b]maximum binding represents the total quantity of radiolabeled detection antibody which can bind to the plate in the system described
[c]< out of low range detection
[d]This particular assay format was applied in O'Connor et al. (25)

Table 7B: Relative cross-reactivities of two site assay using B152 as capture antibody. The molar quantity of ligand required to produce binding equal to 50% of the maximum binding achieved by C5 was determined. Cross-reactivity shown in this table as a percentage is calculated by dividing the molar quantity of the standard by the molar quantity of the other ligand at 50% maximum binding dose.

Ligand	B207[a,d]	B204[a]	B201[a]	B108[a]	B109[a]	A109[a]	CTP104
C5	100%	100%	94%	42%	53%	100%	<
813 CR 127 hCGn	10%	<[c]	<	15%	32%	64%	<
814 CR 127 hCG	7%	<	<	30%	100%	26%	<
hCGβ	6%	20%	19%	11%	<	<	<
C5β	190%	100%	100%	100%	<	<	<
hCGβ core	<1%	<	<	<	<	<	<
hLH	<1%	<	<	<	<	<	<
hLHβ	<1%	<	<	<	<	<	<
hCGα	<1%	<	<	<	<	<	<
Max binding[b]	64%	2%	44%	80%	2%	14%	25%

[a]labeled detection antibodies
[b]maximum binding represents the total quantity of radiolabeled detection antibody which can bind to the plate in the system described
[c]< out of low range detection
[d]This particular assay format was applied in O'Connor et al. (25)

G3 cont

Please amend the paragraph beginning at page 79, line 24, and insert Table 8, as follows:

In order to further explore the nature of the B152 binding site, a commercially available peroxidase-labeled general hCGβ antibody (4001) was employed as a detection antibody in a two-site enzyme immunometric system. Eight different hCG forms were evaluated in this system illustrated in Figure 10. Results are analyzed in terms of relative immunopotency (based on the slope of the regression line) in ~~Figure 14~~ Table 8. Linear regression correlation analysis was performed to compare the relationship of the immunopotencies of preparations 814, C5, M4, C7 and P8 one at a time with the carbohydrate differences (~~Figure 11~~ Table 5) as well as nicking differences among the heterodimeric isoforms of hCG. The correlation results for each comparison are as follows: 1. Tetrasaccharide O-linked core: R^2=0.9147 P=0.0108, significant; 2. Trianntennary branched moieties N-linked on β: R^2=0.3853 P=0.0171, significant; 3. Sialic acid O-linked: R^2=0.3062 P=0.3332, not significant; 4. Sialic acid

G4

N-linked on β: R^2=0.2289 P=0.4149, not significant; 5. Percent nicking in β subunit: R^2=0.0984 P=0.6072, not significant.

Table 8: Immunoreactivity of antigens in the B152 immunoradiometric assay. The dose-response curves used to provide data for this table are shown in Figure 3. Each curve was fitted with 4-5 points. Slope and coefficient of determination (R^2) were determined using a non-linear regression algorithm. Slopes were used as an indicator of antigen potency. Relative potency was estimated as the slope of antigens relative to the slope of C5 choriocarcinoma hCG (the immunogen).

Reagent	Slope[a]	S.E.	R^2	Relative Potency
814 hCG	0.1588	0.0098	0.992	10.2%
C5 chorioCG	1.5603	0.1015	0.983	100%
M4 mole hCGn	0.5317	0.0240	0.992	34%
813 hCGn	0.0986	0.0021	0.999	6.3%
C7 chorioCG	1.4515	0.1246	0.985	93.0%
P8 hCG	0.2192	0.0031	0.999	14.0%
M4 mole hCGβ	0.1038	0.0069	0.991	6.67%
C5 chorioCGβ	0.1286	0.0042	0.998	8.24%
CR 129 hCGβ	only 2 points			
hLH batch I-1	no response			

[a]Slopes are from Figure 3 as calculated in SigmaPlot 4.01 by linear regression analysis. Units of slope are pmol/ml absorbance at 492 nm